

Agriculture polymers: beyond tomorrow

The scientific gains with present state-of-the-art technologies are enhancing industrial demands for agricultural materials. Some analysts expect that by the mid-1990s this demand could double. But what about beyond the year 2000? For these advances to continue, science must probe deeper than ever into the structure-function of proteins, polysaccharides and lipids, natural polymers of agricultural materials, and the basis for functionalities demanded by consumers of industrial products. Development of sophisticated research instrumentation and innovative experimental designs advanced by computer technologies, especially molecular modeling, are allowing researchers to probe deeper into natural polymer physicochemistry. This is leading to new ways of engineering cost-effective processes and high-performance specialty ingredients and products. The innovative research programs shared by the experts participating in the "Agricultural Polymers and Their Use in New Industrial Products" symposium, held Oct. 13-15, 1993, in St. Louis, Missouri, are summarized in this article. These are but a small sample of concepts and ideas evolving in the world of science and technology that will contribute to "Agriculture Polymers: Beyond Tomorrow."

"New and Conventional Approaches to Polysaccharide Modifications," by Manssur Yalpani, examined selected newer methods for preparing and modifying carbohydrate polymers. Substantial progress has occurred in developing specialized modifications based on the discovery of improved solvent systems and unique reactions. A much fuller repertoire of selected chemical derivatization of special functionalities or sites exists; numerous enzymatically catalyzed alterations have been developed. Control of polysaccharide structure, degree of branching and molecular weight permit changes of properties, including viscosity and gel strength. Reactions with glycosidases, proteases and lipases in nonaqueous media for selected

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acylation, esterification, etc., of carbohydrates, by reversing the hydrolytic mode of reaction of aqueous media, have been developed. Engineered proteases, glycosyltransferases and other glycoenzymes, multienzyme systems, and combined chemical-enzyme (chemo-enzymatic) reactions are available for synthesis of carbohydrate polymers. Carbohydrate-derived materials, such as covalent hybrids, cellulose-chitosan, glycan-polyhydroxyalkanoate and alginate-poly(ethylene glycol) derivatives, have been prepared. Physical processes, such as high shear and ultrasound or mediation of supercritical fluid solvents, have resulted in microfibrillar cellulose and chitin crystallites. Combinations of carbohydrate with non-carbohydrate synthons give rise to novel conjugates with other bio- and synthetic polymers or supramolecular structures, such as polyrotaxanes.

Robert B. Freidman in "Starch Modification Through Biotechnology" noted that as users become more skilled in starch application, major differences are observed in the behavior of starch from different biological sources. Hence, skill is needed to determine the appropriate type of starch to select for a particular application. As understanding of starch biopolymer structure-function improves, so do the predictive skills to select specific starch-based materials for defined applications. Starch functionality is intimately tied to structure, which varies among maize genotypes. For example, waxy maize starch has extremely large amounts of the highly branched amylopectin. This starch has strong viscoelastic properties that limit its use. The dull waxy maize, which also has high amylopectin but behaves rheologically as if it were

lightly crosslinked, forms functionally textured gels. The cost to use this starch is less since little or no chemical modifications are needed. A high-amylose starch maize variety was shown to have excellent encapsulation properties. Genetic manipulation via classical breeding methods or genetic engineering has made it possible to select for specialized structural characteristics of starch biopolymers. This permits the cost-effective preparation of biopolymers for specialized uses that require no further modification or minimal chemical treatment.

On "Utilization of Starch in Plastic and Other Industries," Jay-Lin Jane described new ways of replacing petroleum-based products and chemicals with starch biomaterials. Studies are showing that modified starches prepared by various physical, chemical and enzymatic approaches display new unique properties, such as cold-water solubility, hydrophobicity, reduced particle size, increased solid concentrations in pastes and enhanced binding capacity, all desirable properties for industrial uses. Several methods have been developed to prepare granular-cold-water-soluble (GCWS) starch that retains its integrity and displays high solubility and viscosity. These GCWS starches demonstrate a high tensile strength in biodegradable plastics, and as encapsulating agents for agricultural chemicals. An acid-hydrolysis technique has been developed to form small-particle starch granules. Biodegradable polyethylene films containing these components display high tensile strength and reduced film thickness. Aluminum-complexed octenylsuccinate starch has hydrophobic surface properties and, hence, increased compatibility with polyethylene and other hydrophobic plastics. Because of increased crosslinking properties, dialdehyde starches have enhanced adhesion properties.

"Biopolyblends of Pectin and Starch" are being shown by Marshall L. Fishman to form excellent biodegradable films. Recent breakthroughs in understanding pectin

structure-function are helping to open new avenues for the use of these agricultural polymers. Structural features related to shape, size and ability to gel have been verified by membrane osmometry, end-group analysis, high-performance size exclusion chromatography with on-line viscometry and electron microscopy. This work showed that the backbone of the pectin polymer was the shape of a rod or segmented rod, or a combination of the two, thus forming brittle films, unless plasticized. Amylose molecules of starch with linear structures were shown to have better film-forming properties than the highly branched amylopectin. Linear-amylose molecules were predicted to form better films with linear pectin polymers. Films of pectin-starch-glycerol (PSG) proved to be strong, fairly flexible water-soluble structures with tensile strengths comparable to those com-

mercialized. Varying the proportions of pectin, starch and glycerol (plasticizer) components produced a variety of films. It was suggested that further modifications, including laminating with biodegradable hydrophobic polymers, chemically altering starch and/or pectin components, crosslinking, etc., could lead to a broad spectrum of packaging materials.

The ability to integrate a wide range of data/databases extending over many computer systems, sites, disciplines and areas of concentration, through the use of abstraction and representation techniques that emphasize molecular functional characteristics, was the basis for "Engineering Protein Structure and Function: Man vs. Nature," by Michael N. Lieberman. The databases include structural parameters and determination methods, physiochemical properties and *in vitro-in vivo* biochemical functions

for proteins. Significant sources of information for these databases include genetics, biochemistry, biophysics, medicine, etc., that require solutions of an interdisciplinary nature. The challenge is to integrate these diverse data to allow further analyses, modeling and simulation to solve a selected problem. The problem chosen for the work was Factor IX (FIX), an enzyme in the blood coagulation cascade for which natural single-site mutations yield a coagulation deficiency termed hemophilia Bm. The study identified data that mapped the protease domain of FIX. Next, a site that could affect physiological function was identified. The ability to modulate inhibitor/substrate interactions through binding to this site followed by identification of candidates was completed. Spectroscopic measurements, FT-IR (Fourier transform infrared) and CD (circular dichroism)

revealed common patterns of structural responses under varying experimental conditions. These studies were additionally correlated with X-ray crystallographic analyses. The databases have led to the mechanism of action of the disease, potential means for modulating genetic defects and rationally planning site-directed mutation or drug/modulator designs. Continuing experiments are expected to extend the database and concepts used.

A fuller understanding of the molecular basis for the calcium-induced solubility behavior of caseins and computer simulation of protein functionality are the objectives of "Correlations of Molecular Modeling of Caseins with Experimentally Obtained Solubilities: Protein-Salt-Water Interactions for Design of New Uses for Agricultural Products," by Harold M. Farrell and Thomas F. Kumosinski. The attempt was to define and model one simple functionality test for caseins—solubility as a function of calcium ion concentration. To understand calcium-protein interactions, the precipitation and resolubilization of selected caseins were investigated. Analysis of the data indicated that a thermodynamic linkage occurs between calcium binding and salting-out and salting-in reactions. Thermodynamic linkage is based upon the concept that changes of proteins, such as caseins, in an observable physical quantity (i.e., solubility) can be linked to ligand (calcium) binding. From these data, a quantitative thermodynamic mechanism was established for the salting-in and salting-out of casein, and binding free energies of these ligand-induced protein solubility profiles were calculated. Molecular-modeling techniques, such as energy minimization and molecular dynamics, were used to mimic protein-salt-water interactions that explain the salt-induced solubility profiles of α_{s1} -casein. Molecular dynamics examines motion and molecular configuration as a function of time, i.e., dynamic motion of molecules in solution; data contributing to these analyses are derived from NMR (nuclear magnetic resonance), EPR (electron paramagnetic resonance) and

fluorescence spectroscopy. A predicted energy minimized three-dimensional structure, α_{s1} -casein, has led to the discovery of hydrophobic sites responsible for precipitation of the protein and potential salt-binding sites contributing to the salting-in process of added divalent salts. The combination of solution physicochemical experiments and molecular modeling has yielded a powerful multifaceted

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approach to developing a molecular basis for structure-function relationships such as salt-induced solubility profiles. This can be quantitated using thermodynamic linkage in conjunction with nonlinear regression analysis. Predictions concerning the type and amount of protein modification that occurs can be used to increase the desired protein functionality in a rational way through chemical and genetic modifications and computer simulations. The new technology is showing how caseins may be modified as phosphopeptide inhibitors of tartar formation on teeth, films strengthened by chromate for holographic recordings, metal ion carriers used in graph polymerization of leathers and enhanced adhesive properties of biologically applicable cements.

The presentation "Structure-Based Development and Analysis of a Super-Macromolecule: The Application of Molecular Modeling Approaches to the Collagen Fiber Model" by James M. Chen discussed understanding of technologies on structure-function relationships

described previously for collagens by chemical analyses and complex X-ray diffraction data using computer-aided design tools. This includes the ability to study surface contours and interhelical side-chain interactions of computer-modeled structural phototypes that can be reliably extended to native collagen structures. The combination of fast computers, high-resolution three-dimensional graphics and specific modeling techniques has opened exciting capabilities for analyzing and manipulating physicochemical data. Hence, complex proteins, such as collagen, can be analyzed and compared three-dimensionally for stereochemistry, a very important parameter for structure-activity relationships assisting in target-based drug design of therapeutics for collagen-related diseases. A second factor is the triple-helical arrangement of collagen in the fiber. Information on the amino acid sequences, side-chain structures and electrostatic properties as part of molecular models is advancing knowledge on the complexity of native collagen and its interactions with other proteins in the formation of real tissue systems (e.g., skin, bone, cartilage and tendon). This will aid in the future development of artificial collagen-based products. Moreover, studying interhelical side-chain interactions may reveal possible reactive sites for synthetic modifying agents, such as those used in the leather tanning industry. Analysis of the exterior surfaces of the fiber models may depict where certain proteins bind, i.e., proteolytic enzymes (collagenases) known to cleave collagen. Furthermore, data on stabilizing consensus repeat sequences found as clusters along the linear sequence are known to trigger coiling of the three-collagen polypeptides in a zipperlike mechanism into the triple-helical conformation. What has not been shown directly is how specific side chains of one collagen molecule interact with the side chains of neighboring collagen molecules. The collagen fiber models developed here allow the study of intertriple-helical collagen interactions. This is adding to insights on the roles that specific side-chains play in the native collagen structure. Collagen

side-chains with which certain cross-linking compounds interact, such as tanning agents used in leathermaking, can be identified in this model. These efforts are leading to projected new uses for the native, collagen fiber models.

The presentation "Carbohydrate Protease Conjugates: Stabilized Proteases for Peptide Synthesis" by Charles A. Wartchow, Matthew R. Callstrom and Mark D. Bednarski demonstrated how mild, multi-site covalent attachment of carbohydrate-based materials to the surfaces of proteases results in conjugates that are remarkably stable in aqueous solutions and organic solvents at elevated temperatures. For example, carbohydrate protein conjugate (CPC) materials prepared from poly (2-*N*-methacrylamide-2-deoxy-D-glucose) and α -chymotrypsin (Glc-CPC[CT]), trypsin (Glc-CPC[T]) and subtilisin BPN' (Glc-CPC[BPN']) showed remarkable stability in distilled water at 45°C. Glc-CPC[CT] and Glc-CPC[T] retained 100% activity, and Glc-CPC[BPN'] 80% activity after 24 hr. Nonmodified subtilisin BPN', trypsin and α -chymotrypsin retained less than 1% activity at 24 hr. The use of proteases for peptide synthesis using Glc-CPC[proteases] materials was demonstrated successfully, and peptides containing two-eight amino acids in acetonitrile solvent were produced where the native enzymes were inefficient catalysts. A library of stabilized CPC[proteases] materials was developed as potential catalysts for the preparation of larger peptides and proteins. This work could prove to be a useful alternative to established methods for preparing proteins for investigating structure-function and the study of materials-related properties, such as site-directed mutations, unnatural amino acid mutagenesis and expression of artificial genes. Moreover, peptides and proteins traditionally are prepared by solid-phase or solution-phase methods and expression in a foreign host, such as *Escherichia coli* or yeast. The approaches shown in this presentation can overcome difficulties of present techniques, such as inefficient peptide or amino acid couplings and problems associated with

the expression of soluble proteins and peptides and their purification.

U.S. agriculture produces over 16 billion pounds of vegetable oils each year from soybean, corn, cotton, sunflower, flax and rapeseed, a fitting basis for the presentation by Marvin O. Bagby on "Seed Oils—A Storehouse for Industrial Materials." Industrial uses of oils include chemicals, such as plasticizers, stabilizers, emul-

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sifiers, surfactants, esters, nylons and resins. Besides detergents and plastics, products that contain chemicals derived from vegetable oils include lubricants, coatings, corrosion inhibitors, adhesives, cleaners, cosmetics, water repellants and fuels. More often it is advantageous to modify the physical or chemical properties of oils for specific applications. An example is isostearic acid, which when derivatized can be used as textile lubricants, softeners and antistatics, coupling agents and emulsifiers, and greases and synthetic lubricants. Products resembling imported tung oil can be prepared by selected isomerization reactions and used as coatings, resins, ink vehicles and plastics. Eruic acid, from rapeseed oil, is derivatized to amides and amines and used in plastics as antiblocking or antistatic agents to make them less sticky and self-adhering. Polyamides from dimer acids of soybean are used as hot-melt adhesives for shoe soles, book bindings, can seam solders, packaging, flexigraphic inks, moisture-proof coatings, and drip- and sag-resistant

paints. Oleic acid is used in nylon products. The spectrum of specialty chemicals that can be made from vegetable oils can be broadened markedly with discovery of economically competitive processes for converting them to shorter-chain fatty acids or adding functional groups.

The "Molecular Engineering of Lipid Microstructure" was shown by Bruce P. Gaber to be contributing to applications, such as controlled-release compounds, artificial cells and biosensors. The variety of structures and chemistry represented by combining different phospholipid head groups with acylchains of various lengths and degrees of unsaturation is unlimited. Also, lipids are characterized by their ability to self-assemble to microstructures (micelles, liposomes, tubules), adding to the diversity of their uses, especially controlled-release applications, e.g., liposome and encapsulated hemoglobin with extended shelf life. A new class of phospholipid microstructures (polymerizable diacetylenic lipids) placed within films of metal (copper) formed metal-clod liquid tubules having properties of marine anti-fouling paints. Photopolymerization products of diacetylenic lipids to form unilamellar liposomes of varying tubule diameters have further broadened this technology. An exciting advance is the use of atomic force microscopy to image, cut and repair a phospholipid tubule. These results suggest that it may be possible to apply complex molecular-scale patterns to lipid surfaces, patterns which subsequently could be stabilized and reproduced. Lastly, combining the inherent stability of modified silicon surfaces with lipid self-assembly and enzyme-catalyzed chemistry offers a route to the construction of large-scale bio/molecular monostructures, such as "lipid-on-a-chip."

Early polymers, derived from what would now be considered biotechnology, included casein adhesives and plastics, shellac, modified starches, paper and wood products, cellulosic derivatives, textiles such as cotton or wool, rubber and leather. "Polymers from Biotechnology" by Charles G. Gebelein explored new advances in

this field that are redirecting attention from synthetic polymers to agricultural, or renewable, resources. These include new cationic cellulose for cosmetic lotions, creams and hair care products, methyl ether cellulose for water-based paints, ceramic glazes, pigment flocculation, thickening agents and tanning and food additives, and sodium carboxy methylcellulose for detergent formulations, thickeners and coatings. Similar applications are being found in modified konjac, carrageenan and starch. Chitosan, a partial hydrolysis product of chitin, has a wide variety of biomedical, agricultural and cosmetic applications. A polypeptide that allows mussels to adhere to structures has been synthesized. Because this polymer is nontoxic, durable and biocompatible, it is being used as an adhesive for wound closures, bone or dental repairs, and tissue bonding. Collagen, gelatin and a polyelastin are finding their way into the medical and cosmetic fields. Spider silks, made up of a group of polypeptides, have potential use as wound repair fibers, bullet-proof vests, parachutes, machine-washable silk garments, and artificial ligaments and tendons. Recent work has placed the genes for producing the polyester, poly- β -hydroxyalkanoates into higher plants, corn and potatoes. This biotechnologically produced polyester has potential for use in thermoplastics. Many of these biotechnology-derived polymers already show promise as economically viable materials for basic applications and in cosmetic and medically related uses.

Clearly, the program presented increased understanding of, and opened new avenues for, science to probe deeper into the structure-function of natural polymers. To reiterate, substantial progress was achieved in developing more uniform and selected modifications of starches based on the discovery of unique reactions. This includes derivatization of specific functionalities or sites and numerous enzymatically-catalyzed modifications, such as in-chain, branched or terminal residues within starches. Attention is being given to control of polysaccharide structure, degree of branching and molecular weight to

permit alterations of properties including viscosity and gel strength. Reactions are with engineered glycoenzymes, multienzyme systems, chemoenzymatic activities and physical processes that form covalent hybrids, such as cellulose-chitosan, glycan-polyhydroxyalkanoate, alginate-poly (ethylene glycol) derivatives, microfibrillar cellulose and

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chitin crystallites. Genetic studies of maize varieties can lead to the manipulation of starch polymers with structural characteristics for defined applications. Modified granular cold-water-soluble and acid-hydrolyzed small particle starches demonstrate high-tensile strength when used in biodegradable plastics, and as encapsulating agents for agricultural chemicals. Films of pectin-starch-glycerol proved to be strong, fairly flexible water-soluble structures with tensile strengths comparable to water-soluble films already commercialized but containing more expensive components. New information on the subunit structures of pectin and starch is broadening the use of these agricultural polymers.

Databases of structural parameters and analytical methods, physiochemical properties and *in vitro-in vivo* biochemical functions for proteins are being computer-integrated for their analyses, modeling and simulation to solve selected structure-function problems. Molecular-modeling techniques were used to mimic

protein-salt-water interactions that explain the salt-induced solubility properties of α_{s1} -casein. The combination of fast computers, high-resolution three-dimensional graphics and specific modeling techniques has opened exciting capabilities for analyzing and manipulating physiochemical data on collagen systems. Mild, multi-site covalent attachment of carbohydrate-based materials to the surface of proteases results in conjugates that are remarkably stable in aqueous solutions and organic solvents at elevated temperatures. The use of carbohydrate protease conjugates for peptide synthesis was successfully demonstrated, and peptides of varying amino acid sequences in acetonitrile solvent were produced. This work could prove to be a useful alternative to established methods for preparing proteins for studying structure-function and for examining materials-related properties based on genetic techniques.

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The opportunities for formulation of new high-performance specialty ingredients, products and processes for unique industrial uses are unlimited.